DETAILED ACTION

Applicant's arguments filed 08/01/2011have been fully considered but they are not persuasive.

Claims 45-46, 50-52, 60-61, 63-65, 68-71, 87-89, 91, 133-135 are pending. The amendment dated 08/01/2011 has been entered. Claims 63, 91 are withdrawn. Claims 1-44, 47-49, 53-59, 62, 66-67, 72-86, 90, 92-132 are canceled.

Claims 45-46, 50-52, 60-61, 64-65, 68-71, 87-89, 133-135 are under consideration.

Claim Rejections - 35 USC § 112

The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

The rejection of Claims **46, 50-52, 60-61, 64-65, 68-71, 87-89, 133** under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention is withdrawn in view of the amendment dated 08/01/2011have.

The rejection of claims **51, 133** under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention is withdrawn in view of the amendment dated 08/01/2011have.

The rejection of claim **69** recites the limitation "the stem cells" in lines 1-2 is withdrawn in view of the amendment dated 08/01/2011have.

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

The rejection of claims 45-46, 50-52, 60-61, 64-65, 68-71, 87-89 under 35 U.S.C. 103(a) as being unpatentable over **Amit et al** (Developmental Biology, 227: 271-278, 2000) in view of **Mummery et al** (Differentiation, 46: 51-60, 1991); **Eiges et al** (Current Biology 2001, 11:514–518, 2001); **Klug et al** (J Clin Invest, 98(1): 216-224, 1996 (IDS)) is <u>maintained</u> for the reason of record dated 03/30/2011, pages 4-9 as discussed in detail below and also apply to the amended claim 133 and the newly added claims 134-135.

Regarding claim 133, as currently amended as described in the previous office action dated 03/30/2011, page 6, lines 20-23, Mummery (1991) teaches the END-2 feeder cells expressed alpha feto protein (p 53, 2nd column).

Regarding newly added claims 134-135, over the canceled claim 54 of the previous office action dated 03/30/2011, as described in the previous office action dated 03/30/2011, page 6, lines 20-23, Mummery (1991) teaches the END-2 feeder cells (p 53, 2nd column). Therefore, for the reason of record dated 03/30/2011, pages 4-9 and specifically page 6 as discussed above the maintained rejection also apply to the amended claim 133 and the newly added claims 134-135.

Note: Applicants' arguments are rebutted to the extent pertaining to the current rejection as each office action stands its own merit regarding art rejection.

Applicants' arguments. Applicants argue regarding Amit in view of Mummery, Amit discloses culturing human embryonic stem cells (hES) on irradiated mouse embryonic

fibroblasts (MEFs); however, the use of MEFs is taught by Amit to maintain the hES cells in an undifferentiated state, which is not relevant to the use of embryonic cell layers for the differentiation of human ES cells, as presently claimed. Examiner Ton indicated during the telephone interview that Amit was merely cited as a reference to hES cells, and that the skilled artisan would have considered it to be obvious to substitute the mouse EC cells of Mummery with hES cells given an understanding in the art that both cells can give rise to many differentiated cell types. Applicant respectfully disagrees. Applicant previously submitted the distinctions between the EC cells and ES cells, and between mouse and human cells, in Applicant's Response filed on October 26, 2009, for example.

a) Distinctions - EC cells v. ES cells, and mouse v. human cells

Mouse EC cells are derived from malignant teratocarcinomas. They are generally karyotypically abnormal (aneuploid); in other words, they do not carry a normal complement of chromosomes. Applicant directs the Examiner's attention to an abbreviated copy of Appendix C, C-8, Stem Cells: Scientific progress and future research directions -NIHpublication June 2001 http: llstemcells.nih.gov/info/scireport/appendixC.asp (Exhibit 1 attached to Response filed October 26, 2009), which discusses the properties of EC cells and highlights the distinctions between human ES cells and EC cells (including mouse EC and human EC cells). The EC cells are adapted for tumor growth and when differentiated, show an inability to differentiate into well-recognized cell types. Even when transplanted, these cells retain the ability to form teratocarcinomas. EC cells are generally known as the malignant version of ES cells or cells of the inner cell mass (ICM), and generally do not differentiate significantly to a diversity of cell types (see Andrews, P.(2002) Phil Trans R. Soc. Lond. B, 357, 405 - 417, Exhibit 2 attached to Response filed on October 26, 2009). Conversely, ES cells from different species are considered "genetically normal" as are the differentiated cell types that arise from them. ES cells are derived from the ICM of blastocyst stage embryos. Since they are karyotypically normal, when grown in immunodeficient mice, they form well-defined non-malignant teratomas with well-organized tissues representing all the three germ layers indicative of pluripotentiality (Thomson et al 1998 referenced in Andrews, P (2002)). In addition, when mouse ES cells are injected into a mouse blastocyst and the blastocyst is retumed to the uterus, chimeric mice are formed in which most, if not all tissues (including the germ cells), can contain progeny of the donor mouse ES cells. In contrast, mouse EC cells rarely contribute to many cells types in the chimera and additionally, rarely if ever contribute to the germ tissue as do the mouse ES cells. The ability of mouse ES cells to contribute so widely to different cell types either in teratomas or chimeras is a defining characteristic of mouse ES cells that demonstrates their true pluripotent nature (Andrews, supra, as Exhibit 2 attached to Response filed on October 26, 2009). Notably many mouse and human EC cells have limited differentiation capacity or have completely lost their ability to differentiate and have become "nullipotent" (Andrews et aL, Biochemical Society

Transactions 33, part 6, 1526-1530, 2005, Exhibit 3 attached to Response filed on October 26, 2009, especially page 1527, right column, bottom paragraph). Therefore, even human EC cells do not behave in the same manner as human ES cells and have a very restrictive differentiation capacity. Mouse ES and EC cells will generate muscle and mesodermal derivatives whereas human EC cells generally are not capable of mesodermal differentiation (Draper, J.S. et a12002 J. Anat. 200, 249 - 258) (Exhibit 4 attached to Response filed on October 26, 2009). Human EC cells also commonly form the trophoblastic lineage while this does not occur with mouse EC cells (Andrews et aL (2005), supra, Exhibit 3 attached to Response filed on October 26, 2009). The human EC cell line NTERA-2, one of the few human EC cell lines that is able to differentiate, is not capable of mesodermal differentiation and shows no evidence of cardiomyocyte differentiation (Gokhale et aL 2000 Cell Growth Diff 11, 157- 162, referred to in Draper et al (2002) Exhibit 4 attached to Response filed on October 26, 2009) on page 256, left column, bottom of middle paragraph); whereas as shown in Mummery et aL (2002), Circulation 107:2733 - 2740 (Exhibit 5 attached to Response filed on October 26, 2009), hES cells are capable of being differentiated in a controlled and reproducible manner into cells of the mesodermal lineage and to form the cardiomyocytes (see abstract, for example). The response of the mouse EC P 19 cells to differentiation agents that enhance cardiomyocyte differentiation also differs from that of hES cells. The teaching of Skerjanc (cited by the Examiner in the Office Action dated June 15, 2009) highlights the required presence of 0.5-1% DMSO (dimethylsulfoxide) to induce cardiomyocyte formation from mouse EC P19 cells as aggregates in suspension culture. Importantly, DMSO has no effect on the differentiation of hES cells to the cardiomyocyte lineage (see Xu et al, Circulation Research 2002; 91: 501-508, Exhibit 6 attached to Response filed on October 26, 2009; see, e.g., the abstract). Therefore, it would not have been obvious that a differentiation inducing signal would have the same effect across both species and also cell type (e.g., mouse or human, EC versus ES cells). It is also notable that DMSO treatment of human EC cells fails to be an effective inducer of differentiation of human EC cells (see Draper et al. (2002) (Exhibit 4 attached to Response filed on October 26, 2009), page 254). There are also prominent differences in the expression of cell surface markers, such as SSEA1, which is expressed on mouse EC and ES cells but not on human EC or ES cells. Human EC and ES cells also express glycolipids SSEA3 and SSEA4, proteoglycan antigens TRA 1-60, TRA 1-80 and GCTM-2 and protein antigens Thyl and MHC class 1, all of which are not seen on mouse EC or ES cells (Andrews et al. (2005), supra, Exhibit 3 attached to Response filed on October 26, 2009). Accordingly, the physical characteristics and the differentiation profiles of these cell types (mouse EC and human ES cells) are quite different. Observations made with one cell type cannot be extrapolated to another. In fact, the uncontrolled nature of mouse EC cells, particularly in their underlying karyotypic instability and response to differentiation agents make their predictability difficult in comparison to other karyotypically normal cell types such as human ES cells. Even human EC cell types are not able to respond to differentiating factors in a manner that is similar to hES cells, even though human EC cells can be differentiated to some lineages to a limited degree. There is no correlation between the experiments performed in mouse EC cells with that performed in hES cells. When considering the abnormal nature of a mouse EC cell, one skilled in the art would have had no reasonable expectation that conditions applied to mouse EC cells would apply to or operate with hES cells at all. This is because hES cells, although still regarded as a tissue culture artifact (Zwaka T.P. and Thomson J.A 2005 Development, 132, 227 - 233) (Exhibit 7 attached to Response filed on October 26, 2009), are far more representative of a normal pluripotent cell type than a mouse EC cell. For all the above reasons, it is respectfully submitted that the generation of cardiomyocytes from hES cells, which has been demonstrated for the first

time by the present invention, **was entirely unexpected** and therefore unobvious over the art which are directed to mouse EC cells.

(b) Further support to show differences between EC v ES and Human v Mouse

During the telephone interview, Examiner Ton asked that the co-culture/differentiation conditions be evaluated to identify differences in the conditions between the mouse EC cells of Mummery and the human ES cells of the invention. Applicant directs the Examiner's attention to the experimental details provided in the specification, which show differences between EC and ES cells and between mouse and human cells cultured under the same conditions, which further supports the surprising nature of the results obtained when human ES cells are co-cultured with embryonic cells such as END-2 cells and are differentiated specifically to cardiomyocytes. Specifically, both mouse EC cell line PC19 and a human EC cell line GCT27X were used in the experiments described in the examples of the present application, and their differences in both methods of culturing and response to the differentiation highlight the distinctions of these cell types. In Example 4 of the specification (pages 26-32), four cell types were cultured as described. This example compares the responses of mouse ES (mES), human ES (hES), mouse EC (P 19EC) (mEC) and human EC cells (GCT27X) (hEC). All of the hEC, mES and hES cells required "feeders", whereas mEC (P19EC) were feeder independent. This emphasizes the difference between EC and ES cells for the mouse and human since both human EC and ES cell lines require feeder cells.

Response to Arguments. These arguments have been considered but are not persuasive. It is obvious to substitute the PA19 ES cells of Mummery coculture system with the undifferentiated human ES of Amit in order for the feeder END-2 cells to induce differentiation of the undifferentiated human ES cells based on the P19 EC and END-2 coculture system of Mummery.

Prior to the time of the claimed invention, one of skill in the art would have recognized that the same differentiation conditions would produce the same results. For example, **Rohwedel et al** (Cells Tissues Organs, 165: 190-202, 1999 (abstract, IDS) and full copy currently provided] teaches treatment of mouse ES-derived embryoid bodies with 10^{-9} RA induces cardiogenesis after day 5 (p 196, 1st column, 2nd and 3rd paragraph). Similarly, Mummery 1991 teaches 10^{-9} RA induces beating muscle cells of P19 EC cells (p 58, 1st column, under effects of RA on P19 EC cells) and END-2 medium induces beating (cardiac) muscle on days 7-8 on P19 EC cells (p 57, 1st column bridge to 2nd column). Therefore, like RA and END-2 medium induces

differentiation of mouse ES-derived embryoid bodies and P19 EC cells analogously END-2 medium would induce differentiation of human ES cells based on the teachings of Mummery. Moreover, **Reubinoff et al** [Nature Botechnology, 18: 399-404, 2000 (IDS)] teaches human ES cells and human EC cells differentiate in a similar fashion where there are clues from work on human EC cells indicating that similar to embryonic cells of many species, human pluripotent stem cells can be induced to differentiate along specific lineages in response to members of the transforming growth factor-beta superfamily (p 403, 1st column, end of 1st paragraph). Therefore, it would be reasonable to expect that using the Mummery system with human ES cells would result in inducing differentiation of human ES cells.

B. Applicants argue that there is no correlation between the experiments performed in mouse EC cells with that performed in hES cells. When considering the abnormal nature of a mouse EC cell, one skilled in the art would have had no reasonable expectation that conditions applied to mouse EC cells would apply to or operate with hES cells at all. This is because hES cells, although still regarded as a tissue culture artifact are far more representative of a normal pluripotent cell type than a mouse EC cell. For all the above reasons, it is respectfully submitted that the generation of cardiomyocytes from hES cells, which has been demonstrated for the first time by the present invention, <u>was entirely unexpected</u> and therefore unobvious over the art which are directed to mouse EC cells. Applicants argue during the telephone interview, Examiner Ton asked that the co-culture/differentiation conditions be evaluated to identify differences in the conditions between the mouse EC cells of Mummery and the human ES cells of the invention. Applicant directs the Examiner's attention to the experimental details provided in the specification, which show differences between EC and ES cells and between mouse and human cells cultured under the same conditions, which further supports the surprising nature of the results obtained when human ES cells are co-cultured with embryonic cells such as END-2

cells and are differentiated specifically to cardiomyocytes. Specifically, both mouse EC cell line PC19 and a human EC cell line GCT27X were used in the experiments described in the examples of the present application, and their differences in both methods of culturing and response to the differentiation highlight the distinctions of these cell types. In Example 4 of the specification (pages 26-32), four cell types were cultured as described. This example compares the responses of mouse ES (mES), human ES (hES), mouse EC (P 19EC) (mEC) and human EC cells (GCT27X) (hEC). All of the hEC, mES and hES cells required "feeders", whereas mEC (P19EC) were feeder independent. This emphasizes the difference between EC and ES cells for the mouse and human since both human EC and ES cell lines require feeder cells.

These arguments have been considered but are not persuasive. For the same reasons as discussed above it is reasonable to replace the P19 EC cells in the system of Mummery with the undifferentiated human ES of Amit because mouse EC, ES and human EC, ES cells respond similarly to clues of differentiation, where mouse EC and mouse ES commonly respond to RA and human EC, ES commonly respond to TGF-beta family factors is taught by Rohwedel/Reubinoff respectively.

C. Applicants argue regarding the difference between mouse and human, on page 29, Example 4 (f)(i), mEC cells (feeder independent) cultured with END-2 cells aggregated spontaneously and after 7-10 days contained areas of beating muscle. On page 30, Example 4 (f)(iii) hEC cells co-cultured with END-2 cells also aggregated but there was no evidence of beating muscle. This shows that the mouse and human EC cells behave differently. Therefore a citation such as Mummery based on the mouse EC cells cannot be relied upon to predict the response of human cells or at least human EC cells.

These arguments have been considered but are not persuasive. For the same reasons as discussed above it is obvious to substitute the PA19 ES cells of Mummery coculture system

with the undifferentiated human ES of Amit in order for the feeder END-2 cells to induce differentiation of the undifferentiated human ES cells based on the P19 EC and END-2 coculture system of Mummery. This is because **Rohwedel et al** (Cells Tissues Organs, 165: 190-202, 1999 (abstract, IDS) and full copy currently provided] teaches treatment of mouse ES-derived embryoid bodies with 10^{-9} RA induces cardiogenesis after day 5 (p 196, 1st column, 2nd and 3rd paragraph). Similarly, Mummery 1991 teaches 10^{-9} RA induces beating muscle cells of P19 EC cells (p 58, 1st column, under effects of RA on P19 EC cells) and END-2 medium induces beating (cardiac) muscle on days 7-8 on P19 EC cells (p 57, 1st column bridge to 2nd column). Therefore, like RA and END-2 medium induces differentiation of mouse ES-derived embryoid bodies and P19 EC cells analogously END-2 medium would induce differentiation of human ES cells based on the teachings of Mummery. Moreover, **Reubinoff et al** [Nature Botechnology, 18: 399-404, 2000 (IDS)] teaches human ES cells and human EC cells differentiate in a similar fashion where there are clues from work on human EC cells indicating that similar to embryonic cells of many species, human pluripotent stem cells can be induced to differentiate along specific lineages in response to members of the transforming growth factor-beta superfamily (p 403, 1st column, end of 1st paragraph). Therefore, it would be reasonable to expect that using the Mummery system with human ES cells would result in inducing differentiation of human ES cells.

Conclusion

No claim is allowed.

THIS ACTION IS MADE FINAL. Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO

MONTHS of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the mailing date of this final action.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Magdalene K. Sgagias whose telephone number is (571) 272-3305. The examiner can normally be reached on Monday through Friday from 9:00 am to 5:00 pm. If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Peter Paras, Jr., can be reached on (571) 272-4517. The fax phone number for the organization where this application or proceeding is assigned is (703) 872-9306. Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll free).

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